Early response of ray parenchyma cells following wounding in sugi (*Cryptomeria japonica* D. Don) wood.

——Seasonal changes of discoloration and cytological structure——

Tadashi NOBUCHI, Yasumi AKAMATSU, Kenichi SATO,
and Hiroshi HARADA

傷害に対するスギ木部放射柔細胞の初期の反応

——変色と細胞内容物の季節的変化——

野渕　正・赤松やすみ・佐藤　憲一・原田　浩

Résumé

Early response of wood parenchyma cells present at the time of wounding was investigated mainly from the cytological point of view. A couple of superficial mechanical wounds which were made by removing bark was inflicted on the trunk in every season. One of the wounds was kept in open condition to the air and the surface of the other was sealed by an adhesive of epoxy resin immediately after wounding. From the observation of the changes of wood after wounding, following results were obtained; (1) Wood generally showed discoloration following wounding. The extent of discoloration showed seasonal fluctuation. In spring and summer in which the cambium is in the active condition the discoloration extended widely. The extent of discoloration decreased in autumn and showed minimum in winter. (2) Cytological changes of ray parenchyma cell contents were different between spring to summer and autumn to winter. From spring to summer, the first symptom of cytological changes was deformation of nucleus which was followed by the discoloration of cytoplasmic matrix and decrease of reserve substances. In autumn and winter, on the other hand, the discoloration of cytoplasmic matrix was followed by the deformation of nucleus. (3) Comparing the open wounds with the sealed ones, both showed the discoloration of wood and cytological changes of ray parenchyma cell contents. The open wounds showed, however, wider extent of cytological changes and also showed darker discoloration than in sealed condition. (4) From the investigation of the relationship between discoloration and fungi it was considered that the existence of fungi was not the indispensable condition for the discoloration of wood. (5) From the results mentioned above, it was revealed that the xylem necessarily showed discoloration following wounding. One of the causes of discoloration was supposed to be the changes of physiological condition of the tissues and cells following wounding.

要　旨

傷害に対する木部柔細胞の初期の反応を，主として細胞学的な観点から検討した。このため実験的に機械的傷害を与え，傷害を与えた時点で木部を構成していた組織の変化を，各季節において，時間的経過を追って観察した。なお，機械的傷害として1組の剝皮を行い，一方はそのまま放置 (open) し，他方は傷害後ただちに表面をエポキシ樹脂系接着剤で被覆 (sealed) した。得られた結果は以下のとおりである。(1)傷害後木部は一般に変色を示した。変色の広がりは季節により異なった。すなわち春・夏には変色は広範囲に広がり，秋にはやや狭く，冬には最小値を示した。(2)放射柔細胞内容物の変化は，春・夏と秋・冬で異なった。すなわち前者では，核の変形，細胞質基質の変色，貯蔵物質の減少の順に生じたが，後者では細胞質基質の変色の後に核の変形がおこった。(3)open と sealed の両者において，材の変色や放射柔細胞内容物の変化が見られた。しかし open の方が一般に変化範囲が広く，また変色は濃色を示した。(4)変色と菌類との関係を検討したところ，材の変色にとって，菌類の存在は必須のものではないと判断された。(5)以上の結果から，樹幹は機械的傷害に伴い，必ず変色することが明らかになった。また傷害に伴う組織の生理的条件の変化が，変色に必須であると判断された。

1. Introduction

Trees are the long term living plants. They suffer, therefore, many kinds of wounds—physical, chemical, biological and nutritional—in their life time[1]. The responses of the trees following wounding are divided into two categories, that is, the one is the formation of new healing tissues after wounding and the other is the changes of the tissues and cells present at the time of wounding. In this report the latter has been studied because the discoloration following wounding is one of the important problems for the timber utilization.

Many reports have been published concerning the response of the trunk following wounding. Recently, Bauch[2] reviewed the discoloration in the wood of living and cut trees. The studies on the discoloration in the wood have been carried out from different view points. They dealt with the relationship between discoloration and microorganisms[3-5], chemical analysis of extractives[6),7], properties of discolored wood[8-10], and microscopical observation[11),12]. In the course of the studies on the causes of discoloration, physiological and pathological factors are under discussion. It is, therefore, important to clarify which factor influences the discoloration of wood.

In this report some fundamental experiments have been carried out to elucidate the causes of discoloration of wood following wounding. Xylem of the trees suffered artificial mechanical wound was investigated mainly from the cytological point of view. A couple of mechanical wounds was inflicted on the trunk. The one was open to the air—open—and the surface of the other was sealed with adhesive of epoxy resin—sealed—. In the former case, the evaporation of water from the wood and the invasion of microorganisms and the air into the wood take place without limitation, and in the latter the epoxy resin functions to some extent as a barrier zone to the movement of materials and invasion of microorganisms. Comparing two kinds of wounds, it is expected to clarify the relationship between discoloration and microorganisms.

Table 1. Date of wounding and felling

Tree	Date of wounding	Date of felling
A	May 8, May 22, May 29, June 2, June 4, June 5	June 6, 1980
B	June 12, June 26, July 3, July 8, July 10	July 11, 1980
C	Aug. 6, Aug. 21, Aug. 27, Aug. 30, Sep. 2, Sep. 3	Sep. 4, 1980
D	Oct. 2, Oct. 16, Oct. 23, Oct. 27, Oct. 29, Oct. 30	Oct. 31, 1980
E	Dec. 16, Dec. 26, Jan. 6, Jan. 9, Jan. 12	Jan. 13, 1981
F*	Nov. 30	Feb. 2, 1980

* Heating and cooling.

Trees are growing in seasonal rhythm. In this study, the responses of the wood following wounding, therefore, have been investigated in different seasons.

The authors thank the personnel of the Experimental Forest Station of Kyoto University for their co-operation and for providing the necessary wood samples. A part of this study was supported financially by the Weyerhaeuser Company Foundation.

2. Materials and methods

2.1. *Sample trees and wounding.*

Some seedlings planted at the nursery of the Experimental Forest of Kyoto University were used for the experiments. The age of seedlings was 8 years old and the diameter including bark at the ground level was between 5–7 cm. Five to six couples of wounds were inflicted on each sample tree in one month to examine the extent of wounding effect. The days of wounding and felling are listed in Table 1.

Artificial mechanical wounds were inflicted on the trees by a chisel which was disinfected with 70% EtOH. A couple of superficial wounds—$1.5 \times 1.5\ cm^2$—was made by removing bark. After removing bark one of the wounds was kept in open condition to the air and the surface of the other was immediately sealed with an adhesive of epoxy resin. A method of wrapping the wounds by the plastic tape for the protection was reported by Shortle and Shigo[13]. In this experiment, the wounds were not wrapped but sealed. The expoy resin adhered to the cut surface of the wood (Photo. 1). The schematic representation of a couple of wounds in radial view is shown in Fig. 1.

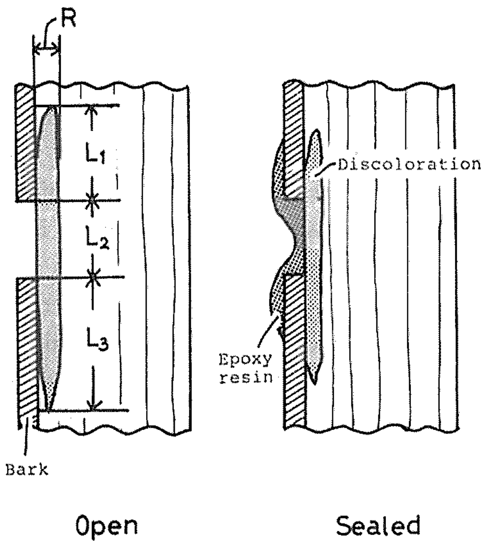

Fig. 1. Schematic representation of open and sealed wounds (radial sections). Notes; R: radial depth of discoloration, L_1, L_2, L_3: longitudinal dimension of discoloration measured.

Some physical wounding treatments—heating and cooling—also were tried. In these wounds the bark was not removed. The bark was heated by the flame of a lighter or cooled by a piece of dry ice in a few minutes. These experiments were carried out only in winter.

2.2. *Observation.*

2.2.1. *Discoloration.*

After felling the radial longitudinal sections were cut at the part of the wound and the extent of discoloration was observed and measured. The measurements were carried out in radial and longitudinal directions with slide callipers (Fig. 2). The extent of discoloration in the longitudinal direction (L) is calculated as $L = L_1 + L_3$ (Fig. 1).

2.2.2. *Light microscopy of cytological structure.*

Wood blocks containing wounds were fixed with 3% glutaraldehyde (buffered with phosphate). Radial sections of about 25 μm thick were cut. The following staining methods were employed; nuclei: safranin and light green, lipid droplets: Sudan IV, starch grains: IKI solution, hypha: safranin and cotton blue, discoloration: no staining.

3. Results and discussion

3.1. *Seasonal changes of discoloration and cytological structure following wounding.*

The extent of discoloration following wounding was large in longitudinal direction, small in radial depth, and slightly larger than the width of the wound in tangential direction. The extent of discoloration, moreover, increased with increasing days after wounding. These results are in accordance with the previous reports[11),14)]. The results of the extent of discoloration in B and D trees are shown in Fig. 2 (a) and (b). The discoloration was observed both in open and sealed wounds. The discoloration in open wound widely extended and generally showed darker color than in sealed wound. The investigation of the relationship between discoloration and chemical analysis of extractives of the wounded tissue is the future research point.

To study the seasonal fluctuation of the discoloration the longitudinal extent of discoloration of 29 days after wounding was plotted in Fig. 3. The longitudinal extent of discoloration showed the maximum value in wounds from June to July (Tree B), gradually decreased towards autumn and showed the minimum value in winter (Tree E). The radial extent showed same tendency as the longitudinal. In winter, the discoloration was surely observed but the only limited area showed discoloration. Ohsako and others[10)] studied the seasonal development of discoloration from the view point of practical use of timbers and reported that the extent of discoloration in the sample trees inflicted wounds in winter was small compared with those in the growing period. From this fundamental experiment, it was revealed that the extent of discoloration was affected by seasonal factors and showed maximum value from June to July. In that period transpiration stream and translocation of assimilation products are in the active condition. It is considered that these factors influence the spread of the stimuli following wounding and determine the distance to which discoloration would progress. The distance to which discoloration progressed was compared in upper and lower part of the wound. The discoloration extended wider in lower part than in upper part. In the separate

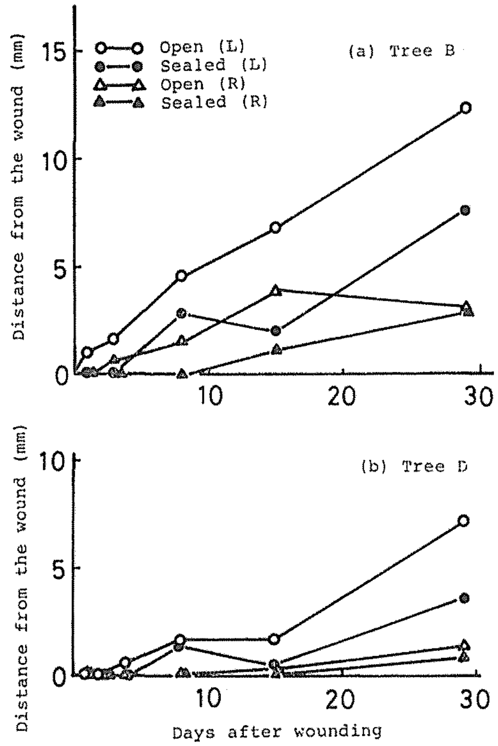

Fig. 2 The extent of the discoloration of wood following wounding. Radial (R) and Longitudinal (L) directions. (a) Tree B, (b) Tree D, in Table 1.

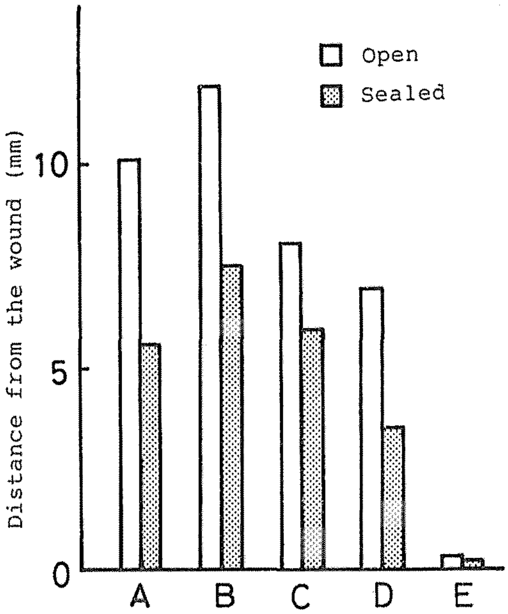

Fig. 3 Seasonal fluctuation of the extent of the discoloration (longitudinal direction). 29 days after wounding. Note; A, B, C, D, E are shown in Table 1.

experiment on the formation of tyloses following wounding in *Castanea crenata*, it was revealed that the distance of tyloses formation was about 50 cm in upper and about 100 cm in lower direction (7 days after wounding in July)[15]. One reason of different development of tyloses between upper and lower directions is supposed to be the water condition in the tree. In the upper part of the wound it is possible that the wood become in a dried condition because of the stop of water supply—stop of the transpiration stream—. The drying of tissues is considered to quicken the necrosis of parenchyma cells before the formation of tyloses and/or discoloration since it needs a certain period to form tyloses and/or discolored substances. In lower part of wound, the necrosis of parenchyma cells would progress following wounding, but the wet condition of the wood is supposed to favor the formation of tyloses and/or discoloration.

Light microscopy of the cytological changes of tissues following wounding was carried out mainly in ray parenchyma cells. In control, a ray parenchyma cell has a large elliptical nucleus (Photo. 2) and small lipid droplets. Starch grains did not distribute near the cambium in the current ring. Nakaue[16] studied the seasonal fluctuation of starch grains and reported that the current ring stored no starch grains from spring to summer and they increased from autumn to winter in the current ring. In sample trees of this experiment, it was the general pattern that the current ring stored no starch grains. The observation of reserve substances was, therefore, carried out mainly in lipid droplets. In this experiment changes following wounding were pursued in radial direction from cambial region to the inner part. Cytoplasmic matrix of ray parenchyma cells in control showed colorless.

The main cytological changes of ray parenchyma cells following wounding were the structural changes and breakage of nucleus, discoloration of cytoplasmic matrix, and decrease of reserve substances. An example of ray parenchyma cells of necrobiosis is shown in Photo. 3. In these ray parenchyma cells, large vacuoles develop and nuclei show the debris-like structure. Some hypha which invaded from outward were sometimes observed. The extent of cytological changes increased with increasing time after wounding in one month experiment. The extent of cytological changes, moreover, fluctuated seasonally and it almost coincided with the extent of discoloration. A regular order was investigated in the changing process of cytological structure, but the order of the changes of cell contents was different depending on the season. The cytological changes of cell contents were measured in every sample tree. The results of June (Tree B) and October (Tree D) are shown in Fig. 4 (a)–(d). In the tree of June, the first cytological change was observed in nucleus which was followed by discoloration of the

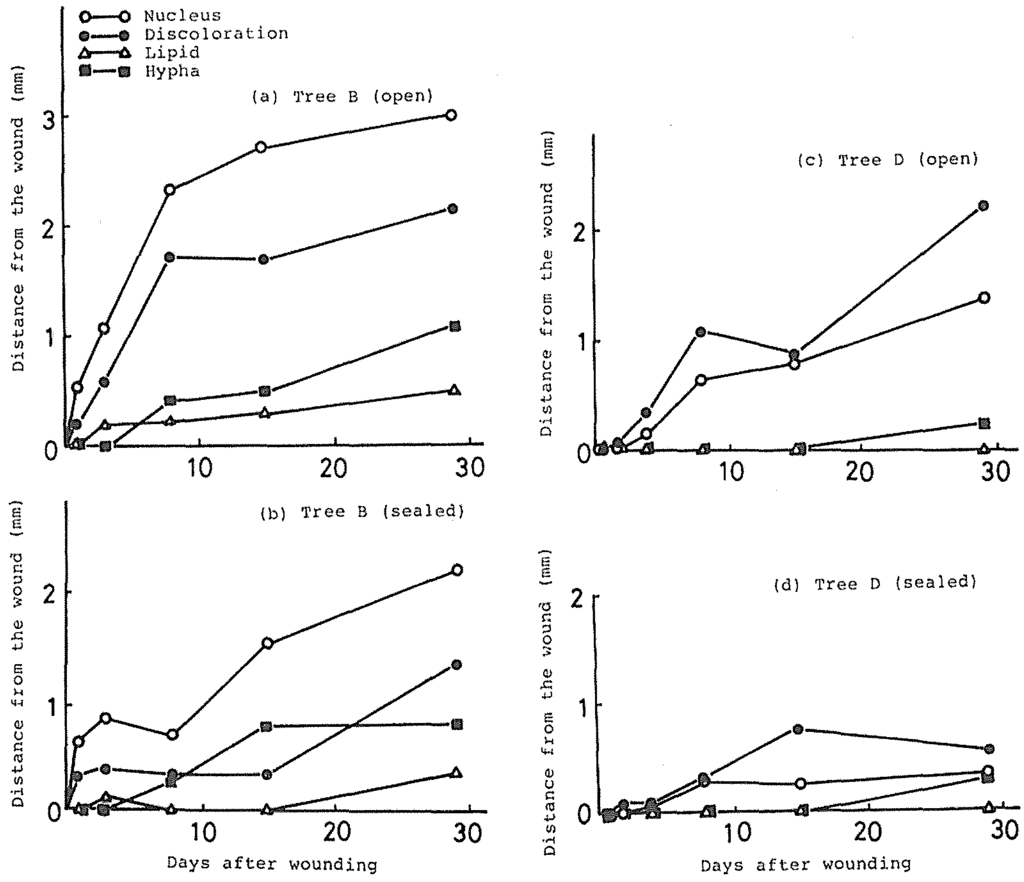

Fig. 4 Cytological changes of ray parenchyma cell contents following wounding (radial direction). (a) Tree B (open), (b) Tree B (sealed), (c) Tree D (open), (d) Tree D (sealed)

cytoplasmic matrix. In October, however, the reverse order was investigated, that is, discoloration of cytoplasmic matrix was the first symptom of changes. The cytological changes of ray parenchyma cells observed are considered to be the indication of necrobiosis. The order of them, however, was different depending on the season. The first change in A, B, and C trees, was the deformation of nucleus, and that in Trees D and E was the discoloration. It is noticeable that the pattern of cytological changes in spring and summer was different from that in autumn and winter. Although it is the future research point to elucidate the cause of this difference, it is considered that the physiological condition of ray parenchyma cells changes seasonally. In autumn and winter seasons, the physiological condition of ray parenchyma cells are in the state which favor the discoloration. From the studies on the seasonal features of heartwood formation it was reported that autumn is the season of conspicuous heartwood formation in warm temperate and temperate zones[17),18)]. Ray parenchyma cells of sapwood were considered to be in the condition that is easy to form discolored substances.

Comparing open and sealed conditions, on the other hand, the extent of discoloration was wider in open than in sealed. Hypha existed generally in open condition and sometimes in sealed condition and this shows the sealing of wound for the protection to fungi was not perfect. The relationship between hypha and discoloration will be described in next section.

3.2. *Relationship between discoloration and hypha.*

It is the important research point that which of physiological or pathological factors affects the wood discoloration following wounding. In this experiment the cause of discoloration was studied comparing the wounds of open and sealed conditions. In the sealed condition it was expected that sealing the wound by epoxy resin would defend the wound part against fungi. In light microscopy, the existence of hypha was attended for the invasion of fungi into wood. Bacteria and other microorganisms were not investigated in this study.

An example of hyphae distributed near the wound is shown in Photo. 4. Hyphae were observed both in ray parenchyma cells and tracheids, but rarely in axial parenchyma cells. The identification of hypha of fungus was not carried out. Light microscopy revealed that the wood of open condition had generally hyphae and a part of sealed condition also had them. This result shows, therefore, that the effect of sealing was not perfect. It is considered that spores and hyphae which attached to the chisel when wounding increased after wounding. The extent of distribution of the hypha was narrower than that of the cytological changes as shown in Fig. 4 (a)–(d). In some instances hypha was not observed at all. In these cases cytological changes and discoloration of wood were observed as in the wood having hyphae. The relationship between hypha and discoloration was investigated (Fig. 5). It was revealed that weak correlation between them was observed in open condition, but the correlation in sealed

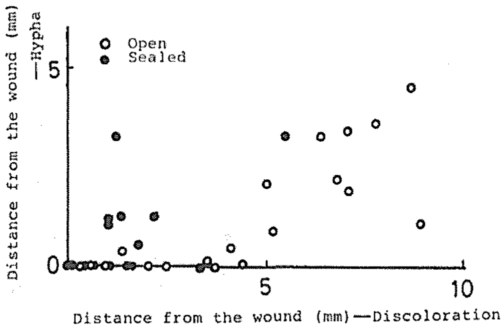

Fig. 5 Correlation between the extent of discoloration of ray parenchyma cells and the distribution of hypha in ray parenchyma cells.

condition was pretty low.

The cytological changes and the distribution of hypha also were observed in the sample trees inflicted the physical wounds—heating and cooling (Tree F). In both cases bark was considered to act as the barrier against hypha because bark was not removed in both cases. In light microscopy no hypha was observed in the physical wounds. The degradation of nucleus, decrease of reserve substrances and discoloration of ray parenchyma cells were, however, observed. An example of changes of nucleus in ray parenchyma cells is shown in Photo. 5. These results revealed that ray parenchyma cells showed necrobiosis and discoloration without the existence of hypha or pathological factor.

From the results in this experiment, it was concluded that hypha was not indispensable factor for the discoloration of wood following wounding. The discoloration of wood was, therefore, derived following mechanical or physical wound, that is, living parenchyma cells sufferred the changes of physiological condition after wounding and tended to necrobiosis. In this necrobiosis ray parenchyma cells showed discoloration and after that wood changed to discolored condition. It is, therefore, considered that the existence of hypha was the secondary factor for the discoloration of wood. The present investigation was carried out in short term experiment (about one month). Trees are the long term living plants. It is supposed that many kinds of wounds affect the trees in their life time. It is needed to pursue the changes of wood, especially discoloration, following wounding in the long period.

References

1) Imazeki, H.: Metabolism of plants inflicted wounds (transl. from Jap.). Kagaku. **40**(5): 226–232, 1970
2) Bauch, J.: Discoloration in the wood of living and cut trees. IAWA Bulletin, New Series. **5**(2): 92–98, 1984
3) Shortle, W. C. and Cowling, E. B.: Development of discoloration, decay and microorganisms following wounding of sweetgum and yellow-poplar trees. Phytopathology. **68**(4): 609–616, 1978
4) Akai, S.: Discoloration and decay of wood of living trees (1). Forest pests. **27**(1): 4–9, 1978
5) Akai, S.: Discoloration and decay of wood of living trees (2). *ibid.*. **27**(2): 21–28, 1978
6) Takahashi, K., Yasue, M. and Ogiyama, K.: Phenols of "tobigusare" wood, discolored sugi (*Cryptomeria japonica* D. Don) sapwood. Mokuzai Gakkaishi. **29**(11): 806–809, 1983
7) Takahashi, K. and Ogiyama, K.: Phenols of discolored sugi (*Cryptomeria japonica* D. Don) sapwood 2. *ibid.*. **31**(1): 28–38, 1985
8) Ohsako, Y., Tsutsumi, T., Nobuchi, T. and Morita, M.: Studies on the forest management from the view point of wood quality. (1) On Botan wood. Bull. Kyoto Univ. Forests. No. 44: 159–175, 1972
9) Ohsako, Y., Tsutsumi, T., Nobuchi, T., and Morita, M.: Studies on the forest management from the view point of wood quality. (2) Development of Botan in connection with pruning. *ibid.*. No. 46, 103–114, 1974
10) Ohsako, Y., Tsutsumi, T., Nobuchi, T. and Morita, M.: Studies on the forest management from the view point of wood quality. (3) Development of Botan in connection with the season of pruning. *ibid.*. No. 50, 69–78, 1978
11) Nobuchi, T.: Light microscopy of the necrobiosis of ray parenchyma cells by wounding—Extent of discoloration in short term experiments—. *ibid.*. No. 47, 152–158, 1975
12) Wardell, J. F. and Hart, J. H.: Early response of sapwood of *Quercus bicolor* to mechanical injury. Can. J. Bot.. **48**(4): 683–686, 1970
13) Shortle, W. C. and Shigo, A. L.: Effect of plastic wrap on wound closure and internal compartmentalization of discoloration and decayed wood in red maple. Plant disease reporter. **62**(11): 999–1002, 1978

14) Yazawa, K., Ishida, S. and Ohtani, J.: A study on the formation of the artificial heartwood. Research Bull. Coll. Exp. Forest Hokkaido Univ.. **25**(1): 9–34, 1967
15) Nobuchi, T., Akamatsu, Y., Sato, K. and Harada, H.: *unpublished*
16) Nakaue, M.: Seasonal fluctuation of reserve substances in the xylem parenchyma cells. Kyoto University graduation thesis: 1978
17) Nobuchi, T., Kuroda, K., Iwata, R. and Harada, H.: Cytological study of the seasonal features of heartwood formation of sugi (*Cryptomeria japonica* D. Don). Mokuzai Gakkaishi. **28**(11): 669–676, 1982
18) Nobuchi, T., Sato, T., Iwata, R. and Harada, H.: Season of heartwood formation and the related cytological structure of ray parenchyma cells in *Robinia pseudoacacia* L.. *ibid.*. **30**(8): 628–638, 1984

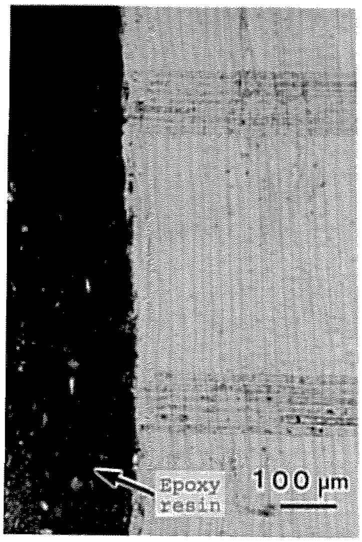

Photo. 1

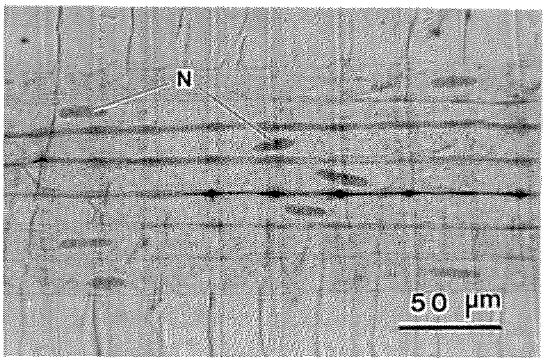

Photo. 2

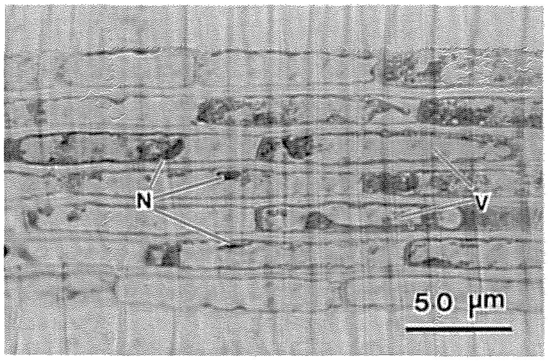

Photo. 3

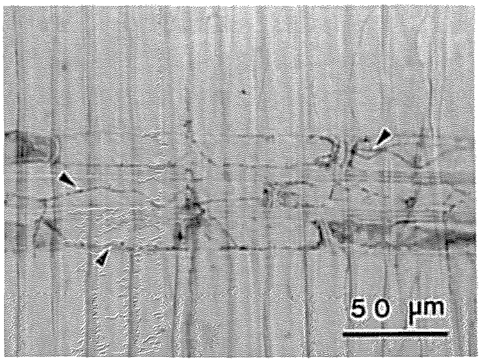

Photo. 4

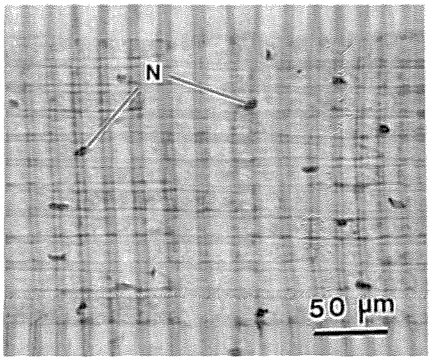

Photo. 5

Photo. 1. Radial section of a portion of sealed wound showing that the epoxy resin attaches to the surface of the wound.

Photo. 2. Radial section of the normal wood showing the long elliptical nuclei in ray parenchyma cells (safranin and light green). Note; N: nucleus.

Photo. 3. Radial section of the wood of open wound showing the necrobiosis of ray parenchyma cells. Large vacuoles develop and nuclei show the debris-like structure (safranin and light green). Note; V: vacuole.

Photo. 4. Radial section showing the hypha (arrows) in the ray parenchyma cells of open wound (safranin and cotton blue).

Photo. 5. Radial section showing the distorted nuclei of ray parenchyma cells which were suffered the physical wound—heating (safranin and light green).